AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph bridging pages 8 and 9 with the following amended paragraph:

Technology has been developed previously to produce highly specific T cell receptors (TCR) which recognize particular antigen. For example, the pending U. S. patent publication US 2007/0116718A1~~applications U.S.S.N. 08/813,781~~ and ~~U.S.S.N. 09/422,375~~ USPN:6,534,633, incorporated herein by reference; and International publications ~~PCT/US98/04274~~ WO 98/39482 and ~~PCT/US99/24645~~WO 00/23087, and references discussed therein disclose methods of preparing and using specific TCRs. Additionally, particular specific TCRs have been produced by recombinant methods as soluble, single-chain TCRs (scTCR). Methods for production and use of scTCRs have been disclosed and are described in ~~pending U.S. patent application 08/943,086~~International application WO 99/18129, ~~and International application PCT/US98/20263~~ which ~~are~~ is incorporated herein by reference. Such TCRs and scTCRs can be altered so as to create fusions or conjugates to render the resulting TCRs and scTCRs useful as therapeutics. The TCR complexes of the invention can be generated by genetically fusing the recombinantly produced TCR or scTCR coding region to genes encoding biologically active proteins to produce TCR fusion complexes. Alternatively, a TCR or scTCRs can also be chemically conjugated with biologically active molecules to produce TCR conjugate complexes.

Please replace the paragraph at page 31, line 11 with the following amended paragraph:

Construction of a shuttle vector has been previously described in ~~pending U.S. application no. 09/422,375~~USPN:6,534,633.

Please replace the paragraph at page 32, line 4 with the following amended paragraph:

After cloning the scTCR, Sc-Fv, linker, and tag DNA fragments into the "shuttle vector" to complete the bispecific sc molecule design, the DNA was restriction digested (AgeI-ClaI) and cloned into the mammalian cell expression vector pSUN27 (Fig. 10) (previously described in International application ~~the pending U.S. Application Serial No. 08/943,086~~ WO 99/18129 to create pBISP/149 (Fig. 11).

Please replace the paragraph at page 32, line 20 with the following amended paragraph:

After cloning the CD3 zeta gene into the "shuttle vector", the DNA was digested AgeI-HpaI to allow for ligation with the 264-A and 264-B scTCR fragments (described above), creating two new scTCR/CD3 zeta fusions. Lastly, the new DNA preparations were restriction digested (AgeI-ClaI) and cloned into the mammalian cell expression vector pSUN28 (pBISP/DO ll. 10 vector), Fig. 11 previously described in ~~pending patent U.S. application no. 09/422,375~~USPN:6,534,633.

Please replace the paragraph bridging pages 34 and 35 with the following amended paragraph:

To create the 149 scTCR (described in detail in ~~patent application 09/422,375~~USPN:6,534,633) version of the IL-2 fusion, the 149 scTCR was cut out of the "shuttle vector" (see example 7 of ~~patent application 09/422,375~~USPN:6,534,633) as an 5'AgeI-3'HpaI fragment and then ligated into the "IL-2 modified shuttle vector" (described above). The 149 scTCR/IL-2 fragment was then restriction digested (AgeI-ClaI) and cloned into the mammalian cell expression vector pSUN28.

Please delete the paragraph on page 11, line 29 to page 12, line 11, and replace it with the following paragraph:

A specific example of a TCR fusion complex fused to an effector molecule is as follows: an sc-TCR such as the p264 sc-TCR disclosed below in Examples 5 below can be produced by transfecting mammalian cells with 264 DNA vector illustrated in Fig. 1. The sc-TCR p264 protein fusion complex recognizes a processed peptide fragment from human wild-type p53 tumor suppressor protein presented in the context of human HLA antigen; HLA-2.1. The sc-TCR p264 and its peptide ligand have been described in Theobald, M.J., et al., *PNAS* (USA) (1995), 92:11993. The peptide sequence is LLGRNSFEV **(SEQ ID NO: 1)**. Expression of tumor suppressor protein p53, is upregulated on malignant cells. It has been shown that 50% of all tumors expressed increased levels of p53 on the surface (Holliston, M.D., et al., *Science*

(1991), 253:49). Therefore, scTCR molecules specific for this epitope could be labeled with a toxin that could than be delivered to the malignant cells expressing the p53 peptide fragment HLA-2.1 ligand. This target specific immunotherapy could be effective at killing only malignant cells. Methods for measuring cytotoxicity *in vitro* are well-known and include conventional viability assays as described below.

Please delete the paragraph on page 12, line 28 to page 13, line 2 and replace it with the following paragraph:

Other suitable effector or tag molecules are known. For example, one tag is a polypeptide bearing a charge at physiological pH, such as, e.g., 6xHIS **(SEQ ID NO: 2)**. In this instance, the TCR fusion or conjugate complex can be purified by a commercially available metallo-sepharose matrix such as Ni-sepharose which is capable of specifically binding the 6xHIS **(SEQ ID NO: 2)** tag at about pH 6-9. The EE epitope and myc epitope are further examples of suitable protein tags, which epitopes can be specifically bound by one or more commercially available monoclonal antibodies.

Please delete the paragraph on page 18, lines 14-23, and replace it with the following paragraph:

Preferred fusion and conjugate complexes in accord with the present invention typically include operatively linked in sequence (N to C terminus): 1) a TCR/one or more linker molecules/ and a biologically active molecule; 2) TCR/ linker molecule /and a biologically active molecule; and 3) TCR/ a first linker molecule /a first biologically active molecule subunit/ a second linker molecule / and a second biologically active molecule subunit. In addition, one or more protein tags such as EE, HA, Myc, and polyhistidine, particularly 6Xhis **(SEQ ID NO: 2)**, can be fused to the N-terminus of the TCR chains as desired, e.g., to improve solubility or the facilitate isolation and identification of the TCR fusion and conjugate complexes.

Please delete the paragraph on page 19, lines 4- 34, and replace it with the following paragraph:

Preferably the linker sequence comprises from about 7 to 20 amino acids, more preferably from about 8 to 16 amino acids. The linker sequence is preferably flexible so as not hold the biologically active peptide in a single undesired conformation. The linker sequence can be used, e.g., to space the recognition site from the fused molecule. Specifically, the peptide linker sequence can be positioned between the TCR chain and the effector peptide, e.g., to chemically cross-link same and to provide molecular flexibility. The linker is preferably predominantly comprises amino acids with small side chains, such as glycine, alanine and serine, to provide for flexibility. Preferably about 80 or 90 percent or greater of the linker sequence comprises glycine, alanine or serine residues, particularly glycine and serine residues. For a TCR fusion complex that contains a heterodimer TCR, the linker sequence is suitably linked to the β chain of the TCR molecule, although the linker sequence also could be attached to the α chain of the TCR molecule. Alternatively, linker sequence may be linked to both α and β chains of the TCR molecule. For covalently linking an effector molecule peptide to a TCR β chain molecule, the amino sequence of the linker should be capable of spanning suitable distance from the N-terminal residue of the TCR β chain to the C-terminal residue of the effector molecule peptide. When such a β+peptide chain is expressed along with the α chain, the linked TCR-effector peptide should fold resulting in a functional TCR molecule as generally depicted in Figure 1. One suitable linker sequence is ASGGGGSGGG (i.e., Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly) **(SEQ ID NO: 3)**, preferably linked to the first amino acid of the β domain of the TCR. Different linker sequences could be used including any of a number of flexible linker designs that have been used successfully to join antibody variable regions together, see Whitlow, M. et al., (1991) Methods: A Companion to Methods in Enzymology 2:97-105. Suitable linker sequences can be readily identified empirically. Additionally, suitable size and sequences of linker sequences also can be determined by conventional computer modeling techniques based on the predicted size and shape of the TCR molecule.

Please delete the paragraph on page 30, lines 4-8, and replace it with the following paragraph:

The T cell clone, 264, recognizes a peptide fragment (aa 264-272; LLGRNSFEV **(SEQ ID NO: 1)**) of the human wild-type tumor suppresser protein p53 restricted by HLA-A2.1. The

T cell receptor gene was cloned into a three domain single-chain format previously shown to produce soluble TCR and functional receptor molecules.

Please delete the paragraph on page 30, lines 21-26, and replace it with the following paragraph:

The alpha and beta chain fragments were cloned into the pGEM-T Easy Vector System (Promega) for DNA sequence determination. Correct fragments were restriction digested and cloned into expression vector pKC60 (described previously in pending U.S. patent application no. 08/813,731) to create two V alpha-$(G_4S)_4$ V beta C beta scTCR molecules $((G_4S)_4$ disclosed **as SEQ ID NO: 4**), 264-A (with V alpha 3) and 264-B (with V alpha 13).

Please delete the paragraph on page 31, lines 14-21, and replace it with the following paragraph:

Briefly, alpha and beta chain TCR fragments were cloned into the into the expression vector pKC60 to create a V alpha-$(G_4 S)_4$ V beta C beta scTCR molecule $((G_4S)_4$ disclosed as **SEQ ID NO: 4**). The new vector was named pNAG2 (Fig. 9). pNAG2 was then reamplified by PCR with primers KC203 and KC208 to generate a 5'AgeI-3'HpaI/BspEI/NruI/ClaI DNA fragment. The scTCR fragment was cloned into the pGEM-T Easy Vector System and this new pGEM-based vector was then used as a "shuttle vector" for introduction of other DNA fragments to create a bispecific sc molecule.

Please delete the paragraph on page 31, line 30 to page 32, line 2, and replace it with the following paragraph:

In the "shuttle vector" design outlined above, a stop codon and splice site were introduced between the NruI and ClaI restriction sites as part of the PCR amplification of the scTCR with "back" primer KC208. To aid in downstream purification of the bispecific sc protein, a set of annealed oligos (KC237 and KC238) was designed to introduce a 3' EE tag (EEEEYMPME) **(SEQ ID NO: 5)** with stop codon and splice site. The annealed oligo pair was

cloned 5'NruI-3'ClaI into the "shuttle vector" already encoding for the complete bispecific sc molecule.

Please delete the paragraph on page 40, line 14 to page 41, line 2, and replace it with the following paragraph:

A DNA preparation of the 264 scTCR provided by Kim Card was used as a template for the PCR amplification of this scTCR construct. Reamplification of the scTCR was carried out using the primer set of 264 TCR1s and KC268. The newly designed 264 TCR1s sequence reads as follows, 5'-TTTCgTACgTCTTgTCCCAgTCAgTgACgCAgC-3' **(SEQ ID NO: 6)**. This oligonucleotide has been designed with a *Bsi WI* restriction endonuclease site and a B6.2 leader. Takara ExTag polymerase was used in the amplification reaction following standard PCR protocol. The amplification profile was as follows, 96oC/2min for 1 cycle; 96oC/30sec, 62C/15sec, 72C/30sec for 5 cycles; and 96C/30sec, 68C/1min for 30cycles. The proper MW (~1.3Kb) DNA band was gel purified following the Clonetech protocol and cloned into Promega's pGem-T easy vector. After ligation and transformation into XL1-Blue cells, six clones were picked and screened by diagnostic PCR using two primers, KC 285 and KC 288, provided by Kim Card. Five clones out of six, produced a DNA band of the proper MW. DNA sequence analysis was carried out on two clones, scTCR264/pGem A and B, with each clone found to be correct. Double digest (*Bsi WI* and *Eco RI*) reactions were set up for clones A and B. The proper DNA fragments were gel purified and pooled together. The purified 264 scTCR was cloned into a previously prepared pJRS355 vector DNA. After ligation and transformation into XL1-Blue cells, two colonies were picked (A2 and B1). An *Alw NI* digest of their DNA showed the proper restriction pattern. Transient transfection using A2 DNA produced a 264 scTCr/IgG1 molecule as determined using an ELISA assay with antibodies specific to the TCR and to the IgG1 isotype.